appear light in color. ''Partially fluorescent'' seeds shall be considered fluorescent. Seeds are considered nonfluorescent if the lemma and palea do not fluoresce and appear dark in color under the ultraviolet light.

[59 FR 64514, Dec. 14, 1994]

EDITORIAL NOTE: For FEDERAL REGISTER citations affecting §201.58a, see the List of CFR Sections Affected, which appears in the Finding Aids section of the printed volume and at *www.fdsys.gov*.

§ 201.58b Origin.

The presence of incidental weed seeds, foreign matter, or any other existing circumstances shall be considered in determining the origin of seed.

[5 FR 35, Jan. 4, 1940. Redesignated at 20 FR 7940, Oct. 21, 1955]

§ 201.58c Detection of captan, mercury, or thiram on seed.

The bioassay method may be used according to the procedure given in Association of Official Seed Analysts, Handbook No. 26, ''Microbiological Assay of Fungicide-treated Seeds'', May 1964.

[38 FR 12733, May 15, 1973]

§ 201.58d Fungal endophyte test.

A fungal endophyte test may be used to determine the amount of fungal endophyte (*Acremonium* spp.) in certain grasses.

(a) Method of preparation of aniline blue stain for use in testing grass seed and plant material for the presence of fungal endophyte:

(1) Prepare a 1 percent aqueous aniline blue solution by dissolving 1 gram aniline blue in 100 ml distilled water.

(2) Prepare the endophyte staining solution of one part of 1 percent aniline blue solution with 2 parts of 85 percent lactic acid ($C_3 H_6 O_3$).

(3) Use stain as-is or dilute with water if staining is too dark.

(b) Procedure for determining levels of fungal endophyte in grass seed:

(1) Take a sub-sample of seed (1 gram is sufficient) from the pure seed portion of the kind under consideration.

(2) Digest seed at room temperature for 12–16 hours in a 5 percent sodium hydroxide (NaOH) solution or other temperature/time combination resulting in adequate seed softening.

(3) Rinse thoroughly in running tap water.

(4) De-glume seeds and place on a microscope slide in a drop of endophyte staining solution. Slightly crush the seeds. Use caution to prevent carryover hyphae of fungal endophyte from one seed to another.

(5) Place coverglass on seed and apply gentle pressure.

(6) Examine with compound microscope at 100–400x magnification, scoring a seed as positive if any identifiable hyphae are present.

(7) Various sample sizes may be used for this test. Precision changes with sample size; therefore, the test results must include the sample size tested.

(c) Procedure for determining levels of fungal endophyte in seedlings from seed samples suspected to contain fungal endophyte:

(1) Select seeds at random and germinate.

(2) Examine seedlings from the sample germinated after growing for a minimum of 48 days.

(3) Remove the outermost sheath from the seedling. Tissue should have no obvious discoloration from saprophytes and should have as little chlorophyll as possible.

(4) Isolate a longitudinal section of leaf sheath approximately 3–5 mm in width.

(5) Place the section on a microscope slide with the epidermis side down.

(6) Stain immediately with the endophyte staining solution as prepared in paragraph (a) (2) and (3) of this section. Allow dye to remain at least 15 seconds but no more than one minute.

(7) Blot off the excess dye with tissue paper. Sections should remain on the slide, but may adhere to the tissue paper; if so, remove and place in proper position on the slide.

(8) Place a coverglass on the sections and flood with water.

(9) Proceed with evaluation as described in paragraph (b) (6) and (7) of this section.

[59 FR 64515, Dec. 14, 1994]